Madison,
Feb. 16, 1952

Dear Cavalli:

It must be equally reassuring to each of us to see such a reciprocal verification of the incredible behavior of the F+ factor. From the first paragraph of your letter of the 28th, I wonder whether I made clear the experiment on transmission of F+. This was first strongly hinted in our similar experiments of "menage à trois". It is more specifically verified by reisolating the transformed F+, e.g., W-1177 (= W-677 S^r) was grown with K-12. The mixture was then streaked out on EMB lactose, and the W-1177 reisolated by the Lac- marker. These colonies, still W-1177 by retest of the other markers now behave as stable F+ in later crosses with W-1607, or other F-. This shows that there is not merely a phenotypic stimulation of the F- (as might explain the menage a trois) but, as you say, an actual "transformation" Like yourself, I have been unable to demonstrate such a transferrence except from viable, intact, F+, cells. (We have certain objections to the term "transformation", and for our Salmonella work have suggested the more expressive term "transduction" for "genetic infection".) In addition to culture filtrates, I ha tried aqueous extracts from dried cells and heat-killed cells, with no success.

Since my last letter, F+ has been shown to be freely transduced from a var of F+ cultures to my two F- testers: W-1607 and W-1177. It is most easily demo strated by inoculating heavy suspensions into Penassay broth. After 1 hour at 37 C., with about 10^8/ml each of an F+ and F-, 10% of the F- were transduced, remarkably high rate of transfer. Under similar conditions, transduction did n occur at 4° nor in synthetic medium with or without the required supplements. Even in longer experiments during which there was considerable growth of both components, transduction of F+ was relatively inefficient in supplemented mini medium, as compared to Penassay, and occurs scarcely at all in unsupplemented minimal, which is useful for further studies. I suspect there is a "phenotypic lag" in the development of F+. Newly transduced cells (in one experiment) did not participate in crosses when tested immediately, although their progenies were F+. This is consistent with the aeration-phenocopy.

I was convinced at first that the phenocopy was due to the rapid growth of the aerated cells (like the attenuation of kappa in Paramecium), but must now don this view in favor of yours. Aeration at 26° which gave growth about as ra as unaerated 37° still gave F- behavior, Furthermore, at 37, an aerated inocul (F-) re-inoculated into aerated broth gave cells that were F+ when harvested at low density, and F- again at maximum growth. I am testing the culture fluid of aerated cultures for activity in suppressing F+ of unaerated cells.* It did not.

There may be, after all, some oppositional character to the F+/F-. In seve combinations, W-1607 (F-) x F+ is more fertile by far than W-1607 (F+) x F+. However, the alternative combinations W-1607 F+ x F- have not shown this high fertility, so that it cannot be ascribed entirely to opposition of F+ and F-. Some F+ stocks, especially when aerated (sic) have been almost sterile with F- but very fertile with corresponding F-! The F-phenocopy is not a general pheno menon, but occurs only with 58-161 and related stocks. The F+ in these cases i the same, as shown by transferring it to W-1607 and W-1177; the different comp bility and aeration responses are due to the rest of the genotype.

Some 7 of our 31 studied wg (interfertile) E. coli are F+ as show by transfer. Some of the F- wg. can acquire F+ from K-12, and transmit it back again, but so far showing no effects on their compatibilities. I have not found F+ so far in non-interfertile strains, but must do more tests. The F+ from these new sources are being transduced back to W-1607 and W-1177 for a closer study of their combinations. I should emphasize again that F+ has been shown to influence fertility only in K-12 derivatives, in agreement with your experience with NTTC 123 (which I confirm as F-, though fertile x K-12 F-). You refer to this strain as self-incompatible. May I ask how you have been able to work with it? We have recently, accidentally, picked up a more or less auxo-autotrophic derivative. Have you secured well-defined auxotroph mutants? I agree with your outlines of the major problems: 1) F+ transduction via cell-free agent; 2) physiological mechanism of the F- phenocopy. To this I would add, 3) the role of F+ in fertility of other strains, and possible differentiation of F+'s, and 4) the possible detection of F+ by other (serological?) methods. Perhaps, for our own purposes, it would not be too soon to consider outlining our joint findings and objectives in the form of a paper.

Sincerely,
Joshua Lederberg

* as titer from colonies from platings at this time